REMARKS

I. Interview with Examiner Yang

Applicants thank Examiner Yang for the courtesy of conducting a telephone interview with Applicants on October 19, 2004. The Kalra and Kononen references of record were discussed, as well as possible amendments to the claims to more clearly define the invention. Applicants noted that, unlike the Kalra reference, the instant invention relates to high-throughput staining involving multiple steps, such as staining used for *in situ* hybridization and immunohistochemical staining. Applicants also thank Examiner Yang for his suggestion that the tissue arrays of the invention be further defined.

II. Support for Amendments

Claims 17 and 21-23 have been amended to more clearly define the claimed invention. Claim 20 has been canceled and its subject matter incorporated into amended claim 17. Claims 24-29 have been added. Support for the amendments is found throughout the specification, for example, on page 3, lines 19-31 to page 4, lines 1-25, and page 9, lines 20-25. Accordingly, no new matter is added by this Amendment and entry thereof is respectfully requested.

III. Rejection of claims 3, 5-8, 11, 12, and 17-23 under 35 U.S.C. § 103(a)

Claims 3, 5-8, 11, 12, and 17-23 were rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kononen et al. (*Nature Med.* July 1998, 4(7), 844-847), in view of Kalra et al. (U.S. Patent No. 5,948,359). With respect to claims 3, 5-7, and 20-23, Kononen is relied on in this Office Action for providing a plurality of tissue microarrays, providing DNA, RNA or protein targets in each of the tissue samples in the arrays, providing and applying

stains that bind to the target molecule *in situ* to the tissue microarrays, and correlating extent of stain binding with clinical utility of the target molecule. Kalra is relied on for teaching that modern laboratories find it desirable to automate the staining process in order to examine large numbers of tissue specimens.

The Examiner states that the deficiency of Kononen is in the failure to specifically disclose that staining is automated in a high-throughput manner. However, the Examiner opines that in view of Kalra and *In re Venner* (120 USPQ 192) (holding that broadly providing a mechanical or automatic means to replace manual activity which has accomplished the same result involves only routine skill in the art), it would have been obvious to use an automated stainer to stain the tissue samples in a high-throughput manner in the method of Kononen. Applicants respectfully traverse this rejection for the reasons discussed below.

To properly make a rejection under 35 U.S.C. § 103, the Examiner has the initial burden of establishing *a prima facie* case of obviousness. Meeting this burden requires the Examiner to show first, that the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process. Second, the Examiner must establish that the prior art would have revealed that in so making or carrying out the claimed process, those of ordinary skill in the art would have had a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be found in the prior art, not in Applicants' disclosure. *In re Vaeck,* 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991), citing *In re Dow Chemical Co.,* 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988).

The present invention is directed to a high-throughput method for evaluating the clinical utility of target molecule(s) using a plurality of tissue arrays. Stains that bind to the target

molecule(s) *in situ* to the tissue microarrays are produced by a multi-step staining process, and applied to tissue samples in a high-throughput manner. Such multi-step staining processes include *in situ* hybridization (ISH) for staining of DNA and RNA target molecules, and immunohistochemical staining (IHC) for staining of protein target molecules.

As Applicants describe in the specification, both ISH and IHC are highly specialized techniques that are complex, time consuming, and vary from lab to lab. IHC requires a series of treatment steps conducted on the tissue array to highlight by selective staining certain morphological features indicative of disease states. These steps typically include: pretreatment of the tissue to remove the paraffin and non-specific binding, retrieval of antigens masked by cross-linking of the proteins, antibody treatment, secondary antibody treatment, substrate reaction, counterstaining, and the like. Most steps are separated by multiple rinse steps. *See* specification at page 3, lines 19-30.

ISH requires a similar series of process steps with many different reagents in order to highlight by selective staining certain nucleotide sequences from the cells of tissue samples. ISH analysis relies on the specific binding of probes with DNA or RNA sequences from the sample cells; as with antibodies, each probe must be individually optimized for reactivity in tissue. Variables that must be optimized include: probe length and labeling, probe concentration, hybridization conditions, stringency washes, and detection morphology. *See* specification at page 4, lines 4-10.

In view of the large number of repetitive treatment steps needed for both IHC and ISH, automated, high-throughput staining is needed to reduce costs and introduce uniformity. The

present invention thus addresses a need on behalf of pharmaceutical and biotechnology companies for performing high-throughput ISH and IHC involving multiple target molecules.

Kalra is relied on by the Examiner for the suggestion that it is desirable for modern laboratories to automate the staining process to examine large numbers of samples. Applicants respectfully submit that the automated staining process of Kalra is unrelated to the high-throughput, multi-step staining process of the present invention. Kalra describes the staining of single tissue samples with single staining reagents. Multiple slides, each generally having a tissue sample at some location on its upper surface, are placed horizontally in a tray in the staining instrument. *See* Kalra, col. 5, lines 55-59. Each of the four trays holds ten slides each, thus the instrument accommodates up to 40 single tissue slides at a time. *See* Kalra, col. 14 at lines 56-60. A typical operating procedure involves the following steps: the movable arm first applies a wash buffer to a slide or group of slides, then applies a staining reagent, then rinses the slides with one or more wash buffer steps. *See* Kalra, col. 6, lines 7-59.

In contrast, the present invention involves the staining of multiple tissue samples with multiple staining reagents. Instead of slides containing single tissues, the present invention involves the analysis of slides containing tissue microarrays of up to 1000 tissue samples each. *See* specification at page 9, lines 5-6. The staining instrument hold up to 20 slides, thus it can accommodate up to 20,000 samples at a time. *See* specification at page 10, lines 26-29. Applicants respectfully submit that the suggestion of Kalra for automating the single-step staining of 40 single tissue samples does not suggest to one of skill in the art that it is possible to automate the multi-step staining of 20,000 samples in a high-throughput manner. Moreover, to the extent that Kalra does suggest automated staining, Kalra does not provide a reasonable

expectation of success for uniformly and efficiently processing 500 times the number of samples disclosed to be the upper capacity limit of the Kalra instrument.

Further, the Examiner relies on the holding in *In re Venner* that broadly providing a mechanical or automatic means to replace manual activity which has accomplished the same result involves only routine skill in the art to support his assertion that automation of staining of the tissue arrays of Kononen would be obvious. Applicants respectfully disagree with the Examiner as to the applicability of this holding to the instant rejection. *In re Venner* applies to the non-patentability of an automatic means to accomplish the same result obtained by manually initiating withdrawal of a core from a piston molding apparatus. 120 USPQ 192, 262 F.2d 91 at 96. In contrast, an automatic means of staining does not accomplish the same result as a manual means of staining because automation allows for high-throughput, uniform, reproducible, and reliable staining, which cannot be achieved manually. A mechanical or automatic means for replacing manual, multi-step staining of up to 20,000 tissue samples does not involve only routine skill in the art. As Applicants previously discussed, both ISH and IHC involve a large number of repetitive and highly specialized treatment steps.

In summary, Kalra does not cure the deficiencies of Kononen. There is no teaching or suggestion in Kononen, alone or as modified by Kalra or *In re Venner*, to apply stains to multiple target molecules by a multi-step staining process, and in a high-throughput manner. Furthermore, Applicants have amended claims 21-23 to more clearly recite stains produced by a multi-step staining process that specifically bind to said at least one target molecule in a tissue sample and to provide an instrument for automatically applying, in a high-throughput manner, the stains to the tissue samples. In view of the above arguments and amendments herein,

Applicants submit that claims 3, 5-7, and 20-23 are not obvious under Kononen in view of Kalra. Applicants respectfully request that the rejection be withdrawn.

With respect to claims 8, 11, 12, these claims are dependent on claims 21 and 23. In view of the above arguments and amendments regarding claims 21 and 23, Applicants respectfully submit that the rejection of claims 8, 11 and 12 under Kononen in view of Kalra have been obviated. Accordingly, withdrawal of this rejection is respectfully requested.

With respect to claims 17-19, Kononen is relied on for describing a tissue microarray having a solid surface with tissue samples mounted to the solid surface. Kalra is relied on for describing the use of slides with bar code labels for optional features that can be included on the apparatus and for optical scanning of slides so that a user is not required to enter information into the computer. The Examiner asserts that it would be obvious to use slides with bar-code labels in the method of Kononen as modified by Kalra so that a user is not required to enter information into a computer. Claim 17 has been amended to more clearly define the tissue microarrays as mounted on a solid surface with a machine readable marking for identifying how the tissues are to be stained with a multi-step staining process, in a high-throughput manner, by a staining instrument.

As Applicants previously discussed, Kononen in view of Kalra does not describe or suggest high-throughput, multi-step staining of tissue microarrays. Kononen in view of Kalra does not describe or suggest tissue arrays to be stained with a multi-step process, in a high-throughput manner, by a staining instrument. In view of the above arguments and the amendments herein, Applicants respectfully submit that the rejections of claim 17, and claims

18 and 19 dependent thereon, under Kononen in view of Kalra have been obviated. Withdrawal of this rejection is respectfully requested.

IV. Rejection of claims 14-16 under 35 U.S.C. § 103(a)

The Examiner has rejected claims 14-16 under 35 U.S.C. § 103(a) as allegedly being unpatentable under Kononen (*Nature Med.* July 1998, 4(7), 844-847), in view of Kalra (U.S. Patent No. 5,948,359) as applied to claims 1-13, and 17-20, and further in view of Bogen (U.S. Patent No. 6,183,693).

Kononen is relied on as described above the description of a plurality of tissue microarrays, providing DNA, RNA or protein targets in each of the tissue samples in the arrays, providing and applying stains to that bind to the target molecule *in situ* to the tissue microarrays, and correlating extent of stain binding with clinical utility of the target molecule. Kalra is relied on for the use of an automated stainer capable of heating. Bogen is relied on for describing a means to heat slides to different temperatures independently of the temperature of the other slides. The Examiner asserts that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use an instrument with multiple heaters in the method of Kononen and Kalra, as taught by Bogen, in order to enhance the rate of chemical reaction during staining. Applicants respectfully traverse this rejection.

Claims 14-16 depend on claim 23. As discussed above, Kononen alone, or as modified by Kalra or *In re Venner*, does not describe or suggest applying stains produced by a multi-step staining process to multiple target molecules in a high-throughput manner, as described in amended claims 21-23. Bogen does not cure these deficiencies. Accordingly, the combination

of the Kononen, Kalra, and Bogen references does not teach or suggest the presently claimed invention. Applicants respectfully request that this rejection of claims 14-16 be withdrawn.

V. CONCLUSION

In view of the foregoing remarks, Applicants believe that the application is in condition for allowance. However, if the Examiner disagrees, he is encouraged to call the undersigned at the number listed below in order to expedite the prosecution of this application.

Respectfully submitted,

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